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EFFECTS OF ATMOSPHERIC PROCESS ON NATURAL ECOSYSTEMS

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EFFECTS OF ATMOSPHERIC PROCESSES ON NATURAL ECOSYSTEMS

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and U.S.D.A. Forest Service Rocky Mountain Experiment Station,
Fort Collins, Co. 80526. - Effects of acid precipitation on reproduction in alpine plant species.

ABSTRACT

A series of experiments were designed to determine the impact of acid rain on plant reproductive processes, a critical component of a species' life history. Research was carried out in herbaceous alpine communities at the U.S.D.A. Forest Service Glacier Lakes Ecosystem Experiments Site in the Snowy Mts. of Wyoming. A range of species were surveyed to monitor the sensitivity of pollen to acidification during germination and growth, and all species demonstrated reduced in vitro pollen germination in acidified media. Field pollinations were carried out in Erythronium grandiflorum and Aquilegia caerulea to determine the reproductive success of plants exposed to simulated ambient precipitation (pH 5.6) or simulated acid precipitation (pH 3.5) prior to pollination. In Erythronium, no differences were observed in seed set and seed weight of fruits resulting from the two pollination treatments. In Aquilegia, fruits resulting the acid spray treatment produced fewer seeds and lighter seeds.

INTRODUCTION

The alteration of atmospheric processes resulting from human activity has become an area of increasing concern in the scientific and political arena (Davis, 1988, Defries and Malone, 1989, Hutchinson and Meena, 1987, Mooney et al, 1987, Pitelka and Raynal, 1989). The evolutionary survival of all species depends on the ability of its members to grow and reproduce under prevailing environmental conditions. Plants exhibit a variety of responses to air pollution, ranging from severe physiological disruption in very sensitive individuals to barely perceptible changes in very tolerant plants (Berry, 1973; Davis and Wood, 1972; Evans and Curry, 1979; Houston, 1974).

Most studies of acid rain effects on plants have focused on direct or indirect injury to vegetative tissues in species of importance to agriculture or forestry (Ferenbaugh, 1976; Hindawi et al, 1980; Evans et al, 1978; Lee et al, 1981). The effect of acid rain on reproductive processes has received much less attention. Acid rain can have indirect and direct effects on plant reproduction. Reproduction may suffer as an indirect consequence of physiological disruption such as chloroplast damage (Ferenbaugh, 1976, Hindawi et al 1980) and root degeneration (Klein, 1984) since reduced photosynthesis and transpiration is likely to affect flower production and fruit development. These effects may underlie the seed yield reductions observed in some crop species under acid stress (Evans et al, 1981). Additional indirect effects of acid rain on plant reproduction include the effects of such acidity on microbial pathogens, nitrogen fixers, and mycorrhizae, and animals that function as pollinators, seed dispersers and predators (Shriner, 1978; Strayer et al, 1981; Bruck et al, 1981; Alstad and Edmunds, 1982).

Acid rain can also interfere directly with reproduction by disrupting fertilization through effects on pollen viability, germination, and tube growth. Angiosperm pollen germination and growth involves a complex series of biochemical interactions between pollen and the stigmatic and stylar tissue (Heslop-Harrison, 1971). Germination and growth of pollen tubes is influenced by the morphology and physiology of pistillate tissue (Heslop-Harrison and Shivanna, 1977; Kroh et al, 1971), and by abiotic features of the environment such as temperature and humidity (Van Herpen 1981; Van Herpen and Linskens, 1981). Pollen germination is very sensitive to changes in pH, and several researchers have reported disruption of pollen growth in response to acid rain (Sidhu, 1983; Wertheim and Craker, 1988; Von Ryan et al, 1986). These studies on the direct effects of acid rain on plant reproduction have also been primarily focused on agricultural species.

Our experiments examine the effects of acid rain on reproductive processes in a natural plant community. Our research site is at an elevation of 3300 meters in the Snowy Mountains of southeast Wyoming. The site lies within the Medicine Bow National Forest, and it has been designated the Glacier Lakes Ecosystem Experiments Site (GLEES). GLEES

is the focus of an interdisciplinary research program carried out by scientists from the Rocky Mountain Forest and Range Experiment Station and a group of university collaborators. The primary thrust of research at GLEES is to identify the effects of global atmospheric changes on this alpine ecosystem. These experiments focus on the direct effects of acidity on pollen germination and reproductive success in alpine plants under field conditions.

MATERIALS AND METHODS

Glacier Lakes Ecosystem Experiments Site is a 300 hectare glacial cirque basin that includes three lakes and the entire watershed surrounding them. The site receives a mean annual snowfall of 2 meters and generally remains snow covered from September through June. Scattered snowfields persist into the month of August. GLEES is also a National Atmospheric Deposition Program site. In addition to this precipitation data, a variety of hydrological and meterological variables such as soil chemistry, solar radiation, relative humidity and air and soil temperature are directly monitored at the site. The landscape patterns at GLEES are diverse, including rock and scree slopes, krummholz, coniferous forest and alpine meadow. This study focused on herbaceous plants from alpine meadows and rocky outcrops.

Two sets of experiments were carried out at GLEES during the months of June through August 1989. The first group of experiments were designed to study the effects of acidity on pollen germination *in vitro* in a range of alpine species. The other set of experiments involved hand pollinations to compare the effects of acidity on *in vivo* reproductive processes in two species, *Erythronium grandiflorum* (Liliaceae) and *Aquilegia caerulea* (Ranunculaceae).

Preliminary germination tests *in vitro* were carried out on freshly collected pollen from twenty one species of alpine and subalpine plants at GLEES. We used standard Brewbaker Kwack media (0.1 g/L boric acid, 0.3 g/L calcium nitrate, 0.2 g/L magnesium sulfate, 0.1 g/L potassium nitrate) (Brewbaker and Kwack, 1963) with a 0.29 M osmoticum (sucrose or Polyethylene glycol (PEG-400)). Trials were also run with the addition of 10^{-3} M Tris to the media (Roberts et al, 1983); pH of all medias used for screening was held constant at pH 5.6. The viability of pollen from all species was assayed using Alexander's stain (Alexander, 1969).

Based on the results of the preliminary germination screening, five species were selected for the study on the effects of acidity on pollen germination *in vitro*. These species were *Erythronium grandiflorum*, *Aquilegia caerulea*, *Lupinus argenteus*, *Castilleja rhexifolia* and *Pedicularis groenlandica*. We acidified the standard Brewbaker Kwack media with a 1:1 (w/w) solution of nitric and sulfuric acid to create experimental medias with pH values of 2.5, 3.5, 4.5, 5.5 and 6.5. A total of 8 - 30 trials were run with each species in all five experimental medias.

Seventy five microliters of each experimental germination media was placed in a depression slide, and 100 - 200 pollen grains of each species were added by lightly contacting the surface of the media with a newly-dehisced anther while looking through a dissecting microscope. The slides were placed on filter paper saturated with distilled water in a closed Petri dish to maintain high humidity. Slides inside these Petri dish "germination chambers" were incubated for two hours at 25 degrees C in a laboratory oven. Samples were examined and photographed under a light microscope, the number of germinated pollen grains was counted, and the condition of pollen tubes in each media was noted. We also carried out a series of trials in which we measured the pH of the media before and after adding pollen to determine the influence of pollen addition on the pH of the media. In these trials one fresh anther of each species was added to 1 ml of each experimental germination media in a plastic multiple well tray. The pH in each well was measured successively for a continous monitoring period of 2-3 hours.

The effects of acid rain on reproductive processes in vivo were examined in Erythronium grandiflorum and Aquilegia caerulea. Flowers of each species were destaminated, tagged and covered with cotton mesh pollinator-exclusion bags. An artificial rain solution was prepared using background ion concentrations obtained from NADP data from GLEES in 1987 and 1988 (Table 1). We adjusted the artificial rain with a 1:1 (w/w) solution of sulfuric and nitric acid to create an "ambient rain" simulant (pH 5.6) and an "acid rain" simulant (pH 3.5). Stigmas of all flowers were misted with either acid rain or ambient rain simulant from hand held spray bottles. The intensity and duration of each spraying was standardized and calculated to result in approximately 4 ml of fluid applied to each flower.

Hand pollinations were then carried out by brushing fresh anthers across each stigmatic surface to assure abundant pollen deposition. In Erythronium, pollinations were carried out 1-2 hours after stigmas were sprayed. In Aquilegia pollinations were carried out approximately 24 hours after stigmas were sprayed. In Erythronium, non-sprayed plants in the same population served as pollen donors. In Aquilegia, out-cross pollen was obtained from non-sprayed flowers on adjacent experimental plants. When possible, crosses were made between the same seed parent and pollen donor in both the acid rain and ambient rain treatment; these are referred to as "matched crosses". After pollination, the plants were covered again with pollinator-exclusion bags, and the fruits were collected when mature. The seeds from each fruit were counted and weighed. Mature fruits were also collected from naturally pollinated Aquilegia and Erythronium flowers that had not been sprayed, and seeds were counted and weighed.

The effect of acid rain on vegetative tissues of Erythronium grandiflorum was also examined. Leaves of 42 plants were sprayed daily for 9 days with our "acid rain" simulant (pH 3.5) and leaves of an additional 42 plants were sprayed daily for 9 days with our "ambient rain" simulant (pH 5.6). The intensity and duration of each spraying was standardized and calculated to result in approximately 4 ml fluid

applied to each leaf. Leaves were collected after 10 days for evaluation of the extent of tissue damage in the two treatments.

RESULTS

The preliminary germination screening tests revealed differences in the germination response of each species in different media formulations (Table 2). PEG functioned well as an osmoticum, supporting the growth of pollen from nearly all species that showed germination in any media, and allowing germination in two species (Vaccinium scoparium and Caltha leptosepala) that didn't germinate in sucrose media formulations. Germination in PEG media was superior to germination in sucrose media in five species.

Significant variation in germination percentages of pollen growing in vitro at different pH values was found in all five species studied (Table 3). Most species showed a marked decrease in germination percentage between pH 4.5 and pH 3.5. The majority of tubes that formed in media below pH 4.5 were irregular and coiled (Figure 1 - Photographs). Pollen of all species failed to germinate below pH 2.5. Aquilegia and Pedicularis showed relatively greater sensitivity at pH 3.5 (1-2 % germination) than the other three species (27-30 % germination).

Pollen of all five species exhibited some ability to modify the pH of the germination media, although the strength of this ability varied among species (Table 3). Erythronium pollen exhibited a stronger buffering ability than than other species studied, tending to decrease the pH of experimental medias at pH 6.5 and 5.5, and increase the pH of medias at pH 2.5, 3.5 and 4.5.

The effects of simulated acid rain on reproductive processes in vivo differed in the two species investigated. In Erythronium there was no difference in the number or weight of seeds produced by plants in the ambient or acid spray treatment. There was also no difference in the number or proportion of aborted seeds. (Table 4) In addition the seed set of hand pollinated plants under both spray treatments was significantly greater than the seed set of non-sprayed plants under natural pollination conditions. No visible damage was found on Erythronium leaves sprayed with acid or ambient rain simulant. There were scattered necrotic areas on a few leaves from both treatments, but due to the lack of no discernable differences between the treatments, leaf area damage measurements were not made.

In Aquilegia, flowers sprayed with acid rain simulant produced significantly fewer seeds and significantly lighter seeds than flowers in the ambient spray treatment (Table 5). These differences were particularly striking in matched crosses (Table 6). Fruits from the acid spray treatment also produced greater proportion of aborted or unfertilized seeds per fruit (Table 5). Flowers from both spray treatments produced fewer seeds than non-sprayed naturally pollinated

flowers from the experimental plant population, although the difference was greater between fruits from the acid treatment. Non-sprayed naturally-pollinated flowers produced seeds that were heavier than seeds from the acid spray treatment, but lighter than those from the ambient spray treatment.

DISCUSSION

Investigations of pH effects on pollen germination _in vitro_ have generally not been designed to include possible effects of germinating pollen grains on the pH of the media. Proton co-transport of organic solutes, particularly sugar, has been recognized in bacterial, fungal and plant cells (Baker,1978). Deshusses et al (1981) found that proton symport results in an increase in ph during sugar uptake in pollen of _Lilium longiflorum_, and they suggest that this proton-sugar co-transport system is important in mobilizing carbohydrates in the pistil exudate. At this point it is unclear whether this proton symport mechanism exists in pollen of other species. It may be a characteristic associated with hollow-styled spècies, or function more generally in aiding pollen utilization of stylar nutrients during tube growth. In any case, the possibility of proton symport mechanisms suggests that sucrose should be avoided as an osmoticum in studies of pH effects on pollen. For this reason, we included an investigation into the use of polyethylene glycol (PEG) in our pollen germination screening studies. PEG has been used widely as an inert osmoticum in plant tissue studies, and it has been used successfully as an osmoticum for the germination of pollen in _Petunia hybrida_ and _Brassica oleracea_, but it didn't support the growth of _Lilium longiflorum_ pollen (Zhang and Croes, 1982; Roberts et al, 1983; Dickinson, 1968). Our results suggest that PEG-400 supports the germination of a wide range of species, and should be considered for use in any experiment where pH change is a critical factor.

Results from the pollen germination screening studies also suggest that Tris is a useful addition to the standard germination media for some species. Roberts et al (1983) reported enhancement of germination in _Brassica oleracea_ by the addition of 1mM Tris. This enhancement does not appear to be a function of pH changes caused by adding Tris since the enhancement is seen in medias over a range of pH values (4-9), and a similar enhancement is not observed when pH levels are changed with NaOH. It is more likely that Tris serves some nutrient function; Roberts et al reported a similar enhancement by methylamine. Polyamines produce significant effects on germination of pollen in vitro, and they are are synthesized during pollen growth (Speranza et al, 1982).

The pollen buffering studies indicate that pollen from all species is capable of changing the pH of media. All species acidified the media initially at pH 6.5, and all species but _Erythronium_ raised the pH of most medias at lower initial pH values. Roberts et al (1983) report that pollen eluates of _Brassica oleracea_ are in the pH range 6.0-6.5. Our results seem to suggest a similar range of pH values for pollen of all species except _Erythronium_, whose pollen appears more

acidic. Southworth (1983) reported pH changes of half to a full pH point during pollen germination in Lilium longiflorum ; media with initial pH values of 2.75 - 5.1 became less acidic and media with initial pH values of 5.8 to 9.9 became more acidic. These pH changes were observed in media containing sucrose and pentaerythritol,(a non-metabolizable sugar). We observe a similar pattern of pH change in our studies of Erythronium, using media with a PEG osmoticum. The degree of pH change resulting from pollen of Erythronium is considerably stronger in than in the other species we studied. The similarity between the response in Lilium and Erythronium may reflect their taxonomic relatedness, but this question cannot be resolved without further studies of genera in the Liliaceae.

Studies in the legume Leucaena leucocephala raise some intriguing questions about the physiological role of pH in pollen germination (Ganeshaiah and Shaanker, 1988). Pollen was germinated in media with sucrose osmoticum and stigmatic extract at two initial pH levels (4.9 and 8.5). The germinating pollen grains buffered both medias to result in a final pH value of 6.5, which is in the optimum range for pollen germination in this species (6.0 -6.5), and is higher than the pH of the stigma (5.0-5.3). Further studies suggest that in this species pollen germination is inhibited by a pH-sensitive protein that is destroyed if a sufficient number of pollen grains (>20/stigma) are present to raise the stigma pH.

Although all species showed significantly decreased germination at low pH values, the strength of inhibition varied among the species. Pollen of Aquilegia and Pedicularis shows greater sensitivity than the other three species at pH 3.5, decreasing by 98% and 95% respectively of their maximum germination percentage. Germination percentage at pH 3.5 in the other species only decreases by a factor of 25% to 57%. These differences in sensitivity couuld result in quite different probabilities of fertilization under natural conditions, since pollen may not be limiting reproduction in many cases, therefore a 25 % reduction in germination may still allow full seed set. Any factor that results in decreased germination percentage will reduce the opportunity for competition between pollen grains however, which could lower average offspring fitness (McKenna and Mulcahy, 1983; McKenna, 1986; Mulcahy, 1979) Cox (1986) compared the pH sensitivity of pollen growing in vitro from a group of twelve species from various habitats in eastern Canada. He found that pollen from broadleaf trees and understory flora (Oenothera parvifolia, Maianthemum canadense, Trillium grandiflorum, Diervilla lonicera, Prunus pensylvanica) was more sensititive to low pH than pollen from the conifers sampled. Cox reported inhibition thresholds for pollen from most broadleaf trees and understory flora to be pH 3.6 - 4.6, while conifers had thresholds ranging from pH 2.6 - 4.6 or 2.6 - 3.6). The pH of ambient rainfall at the GLEES site ranges from pH 4.63 - 5.49 during the flowering season, so these results do not indicate any danger to the species studied under current levels of acid pollution. However, pH values within the range shown to be inhibitory to the GLEES species (pH 3.5-4.5) are common in much of the northeastern United States and parts of Europe (Wellburn, 1988).

The seed set data from the pollination studies indicates that reproductive processes in vivo in Erythronium are less sensitive to acidity than reproductive processes in Aquilegia. This result corresponds with the differences in sensitivity seen in pollen germination studies in vitro. Lower seed set of Aquilegia flowers sprayed with acid rain simulant may reflect poor pollen growth in vivo; this possibility is being explored through fluorescence microscopy studies currently underway. A possible explanation for the difference in response of these two species may relate to differences in phenology and habitat. Erythronium is an early snowmelt species ; buds are formed while plants are still under snow. In contrast, Aquilegia is found on well drained soils of rocky ledges and blooms later in the season. Erythronium may experience a more acidic environment since snow concentrates airborne pollutants in insoluble form that become soluble as the snow melts, releasing a pulse of acidity. Thus the difference in these two species' relative sensitivity to the acid spray treatment may reflect selection for survival in different environments.

Hand pollinations in Erythronium following both acid and ambient spray treatments resulted in greater numbers of seeds per fruit than found in open pollinated non-sprayed controls. This suggests that reproduction may be limited by the availability of pollen under natural conditions. Casual observation of pollinator activity indicates that a depauperate pollinator fauna may be at least partially responsible for the relatively lower seed sets in controls. In Aquilegia, open pollinated non-sprayed controls produced greater numbers of seeds than hand pollination of either the acid or ambient sprayed flowers. In this case, both spray treatments may have interfered with the fertilization process. It is also possible that ovule number may vary throughout the season in this species. Since the open pollinated flowers were pollinated later than the hand pollinated flowers, the differences in seed number may reflect increased ovule number in flowers formed during this period. These questions will be addressed in further studies at the GLEES site.

The long term effects of differential sensitivity to pH change among these alpine species can only be explored through experiments designed to evaluate the plants throughout the entire growing season. Funk and Bonde (1986) reported a differential reproductive response in field populations of Acomastylis rossii and Bistorta vivipara sprayed with nitric/sulfuric acid mist. Flower production was reduced in Acomastylis, while Bistorta showed an increase in flower production and a decrease in asexual bulblet production. Long term studies such as these with particular emphasis on phenology, habitat, and floral morphology are currently being developed for implementation during Summer 1990.

Studies on the effects of acid rain on pollen growth are also of interest from another perspective. Studies in many species suggest that there is an extensive overlap in genotypic and phenotypic expression between pollen and the diploid plant (Searcy & Mulcahy,1986; Sacher et al, 1983; Smith and Moser, 1985; Tanksley et al, 1981; Sari-Gorla et al, 1986; Weeden, 1986). The overlap in genetic expression in the gametophyte and the sporophyte suggests that one may be able to use

pollen systems to predict sporophytic response to air pollution stresses. Evidence from the limited studies in this area so far indicates that the sensitivity to acid rain of pollen germinating in vitro is closely correlated with the sensitivity of the pollen parent in Oenothera parvifolia (Cox, 1984). Thus pollen sensitivity tests may prove useful in identifying particularly resistant and vulnerable species. Screening for sensitivity using pollen systems requires little space and offers the advantages of speed, economy and efficiency. In addition, the simple morphology of the pollen grain allows one to test for true physiological stress tolerance without complications arising from the stress avoidance mechanisms that may be present in the multicellular plant. Studies relating sporophytic and pollen sensitivity to pH in the species examined so far are also being developed for implementation during Summer 1990.

These studies suggest that acid rain may significantly impact the reproductive success of plants in the alpine community, and that the response to levels of acidity varies among species. The effects of acid rain on plant reproduction may be subtle, yet can lead to long term community alteration, including changes in species composition and diversity. Alpine plant communities are diverse, reflecting the wide diversity of microhabitats in the alpine environment, ranging from exposed scree slopes to wet meadows (Bliss, 1971). Complex ecological relationships exist between alpine plant species and other biotic (living) and abiotic (physical-chemical) features of of the alpine ecosystem (Tranquillini, 1964). Human alteration of the atmospheric component of this ecosystem is likely to result in widespread impacts on these relationships. For example, a shift in species composition following the removal of sensitive species may alter competitive interactions (Materna, 1984; Smith, 1974) and disrupt plant interactions with soil microorganisms such as nitrogen fixing bacteria (Shriner and Johnston, 1981) and mutualistic fungi (Klein, 1984). Studies to determine the extent to which alpine plants might suffer reproductive losses due to acid rain and other atmospheric changes are important, since these reproductive losses carry ecological and evolutionary implications for the alpine ecosystem. These studies must also be interpreted from an ecosystem perspective since many factors may interact to produce the observed patterns. For example, the response of plants to acid rain may depend on the presence of other interacting stress factors such as drought, frost, predators, pathogens, and nutrient stress.

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TABLE 1. NADP - GLEES Precipitation Chemistry (June 1988, July and August 1987). Values are mg/L.

	JUNE (n=3)	JULY (n=4)	AUGUST (n=4)	Y	s	n
PH	4.77	5.06	5.01	4.97	0.252	11
Ca	0.32	0.17	0.11	0.189	0.123	11
Mg	0.038	0.023	0.019	0.026	0.015	11
K	0.033	0.015	0.011	0.018	0.020	11
Na	0.094	0.047	0.071	0.069	0.033	11
NH_4	0.063	0.10	0.011	0.084	0.052	11
NO_3	1.57	0.70	0.83	0.984	0.416	11
Cl	0.10	0.08	0.10	0.102	0.037	11
SO_4	1.44	0.64	0.73	0.893	0.518	11

TABLE 2. Germination percentage of all species used in preliminary screening study in each media.

SPECIES	MEDIA			
	BK-SUCR	BK-TRIS-SUCR	BK-PEG	BK-TRIS-PEG
Polemonium viscossum	6	29	30	23
Penstemon whippleanus	69	0	55	0
Erythronium grandiflorum	13	-	51	50
Pedicularis parryi	31	67	45	2
Pedicularis groenlandica	38	0	2	53
Caltha leptosepala	0	0	48	72
Phacelia sericeus	56	29	30	43
Phlox sibirica	52	56	31	34
Castilleja rhexifolia	64	51	29	40
Trollius laxus	44	40	0	0
Lupinus argenteus	-	61	-	87
Aquilegia caerulea	80	80	80	80
Castilleja sulphurea	-	95	-	60
Vaccinium scoparium	0	0	28	0

The following species showed no germination in all four medias tested: Kalmia polifolia, Claytonia lanceolata, Salix planifolia, Geum rossii, Potentilla diversifolia, Ribes montigenum, Erigeron melanocephalus

TABLE 3. Mean pollen germination percentages of each species at each pH level. Values in parentheses are the mean number of grains per sample. Final pH values are based on measurements taken after 2 hours in a separate pollen- buffering study. Kruskal-Wallace test was used to compare germination percentages within each species.

ERYTHRONIUM GRANDIFLORUM (BK - PEG Media); N = 14

Initial pH	Final pH	% Germination	Test Statistic	p
6.5	5.7	69.3 (139)	84.58	.001
5.5	5.3	64.5 (120)		
4.5	5.0	50.4 (172)		
3.5	3.9	29.6 (88)		
2.5	2.7	0.0 (229)		

AQUILEGIA CAERULEA (BK - Tris/PEG Media); N = 30

Initial pH	Final pH	% Germination	Test Statistic	p
6.5	6.3	57.9 (175)	102.71	.001
5.5	5.7	66.5 (176)		
4.5	4.8	69.4 (177)		
3.5	3.6	1.4 (157)		
2.5	2.6	0.0 (130)		

LUPINUS ARGENTEUS (BK - Tris/PEG Media); N = 8

Initial pH	Final pH	% Germination	Test Statistic	p
6.5	6.4	54.1 (90)	15.56	.01
5.5	5.7	62.2 (81)		
4.5	4.5	56.1 (76)		
3.5	3.4	30.1 (97)		
2.5	2.5	6.0 (179)		

CASTILLEJA RHEXIFOLIA (BK - Sucrose Media); N = 8

Initial pH	Final pH	% Germination	Test Statistic	p
6.5	6.3	26.0 (118)	19.16	.001
5.5	5.5	36.4 (166)		
4.5	4.7	31.3 (160)		
3.5	3.5	27.4 (170)		
2.5	2.7	0.0 (148)		

PEDICULARIS GROENLANDICA (BK - Tris/PEG Media); N = 8

Initial pH	Final pH	% Germination	Test Statistic	p
6.5	6.3	39.7 (103)	20.78	.001
5.5	5.8	36.1 (91)		
4.5	4.7	31.6 (151)		
3.5	3.5	1.9 (139)		
2.5	2.5	0.0 (126)		

TABLE 4. Comparison of reproductive output in Erythronium fruits from natural pollinations in nonsprayed plants and from hand pollinations of flowers sprayed with acid rain simulant (pH 3.5) or ambient rain simulant (pH 5.6). Means were compared with the Mann-Whitney U test; means with different letters are significantly different at $p < .05$ level.

ERYTHRONIUM GRANDIFLORUM

	Ambient Spray	Acid Spray	Non-Spray Control
SEEDS PER FRUIT	Y = 37.13 a s = 18.24 n = 23	Y = 39.27 a s = 20.68 n = 22	Y = 32.49 b s = 13.68 n = 39
TOTAL WEIGHT OF SEEDS PER FRUIT (mg)	Y = 137.9 a s = 69.9 n = 23	Y = 145.4 a s = 86 n = 22	Y = 151.3 b s = 83 n = 39
MEAN SEED WEIGHT (mg)	Y = 3.72 a s = 0.87 n = 23	Y = 3.76 a s = 1.15 n = 22	Y = 4.69 b s = 1.50 n = 39

TABLE 5. Comparison of reproductive output in Aquilegia fruits from natural pollinations in nonsprayed plants and from hand pollinations of flowers sprayed with acid rain simulant (pH 3.5) or ambient rain simulant (pH 5.6). Means were compared with the Mann-Whitney U test; means with different letters are significantly different a $p < .05$ level.

AQUILEGIA CAERULEA

	Ambient Spray	Acid Spray	Non-Spray Control
SEEDS PER FRUIT	Y = 60.70 a s = 44.65 n = 10	Y = 36.33 b s = 54.73 n = 12	Y = 67.88 c s = 52.71 n = 68
TOTAL WEIGHT OF SEEDS PER FRUIT (mg)	Y = 48.2 a s = 39.3 n = 10	Y = 20.5 b s = 27.5 n = 12	Y = 41.0 c s = 29.7 n = 68
MEAN SEED WEIGHT (mg)	Y = 0.70 a s = 0.10 n = 10	Y = 0.58 b s = 0.13 n = 12	Y = 0.63 c s = 0.24 n = 68
% UNFILLED SEEDS	Y = 34.3 a s = 24.3 n = 10	Y = 57.5 b s = 32.2 n = 12	Y = 54.5 b s = 26.2 n = 68

TABLE 6. Comparison of reproductive output in Aquilegia fruits from hand pollinations of flowers sprayed with acid rain simulant (pH 3.5) or ambient rain simulant (pH 5.6) in crosses between the same seed parent (S.P.) and pollen parent (P.P.).

AQUILEGIA CAERULEA (MATCHED CROSSES)

SEEDS/FRUIT

S.P.	P.P.	Ambient	Acid
39	3	42	16
39	3	52	35
1	35	53	2
4	28	98	56
10	26	16	6

% UNFILLED SEEDS

S.P.	P.P.	Ambient	Acid
39	3	41	65
39	3	36	73
1	35	41	93
4	28	26	34
10	26	59	85